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# A NOTE ON THE SUSCEPTIBILITY OF SEGMENT- ING ARBACIA AND ASTERIAS EGGS TO CYANIDES.<sup>1</sup>

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The discovery by Lyon<sup>2</sup> that after fertilization sea-urchin eggs show definite periods of resistance and susceptibility to the action of cyanides is of such general interest that it is important to establish the exact period at which eggs are most sensitive and to discover whether other eggs also show similar periods of susceptibility. Lyon found that the eggs were most easily killed about the time of the first cleavage. He was in doubt as to the exact period, but thought it came just after the cleavage, whereas immediately preceding cleavage the egg was very immune. He made no sections and was not entirely certain with just what processes in the egg these periods of immunity and susceptibility coincided. Spaulding<sup>3</sup> tried similar experiments using ether and acids. The ether especially gave a very sharp result and showed great susceptibility immediately *preceding* segmentation. The acid, as might be anticipated from the various actions it possesses, gave a far more complicated series of phenomena. It seemed desirable to ascertain exactly the period of susceptibility to the cyanides and to discover if possible what series of changes within the egg corresponded with the periods of susceptibility and immunity. A study was accordingly made of the living eggs of *Arbacia* and *Asterias* and I have reëxamined a series of sections of *Arbacia* eggs made several years ago, in which the eggs were preserved in sublimate-acetic at definite intervals after fertilization. I also endeavored to repeat Lyon's observations on *Arbacia* using *Asterias Forbesii* (?) eggs. Some very fine material was gathered about the middle of September at Woods Holl.

<sup>1</sup> Laboratory of Biochemistry, University of Chicago, and the Marine Biological Laboratory, Woods Holl.

<sup>2</sup> Lyon, *American Journal of Physiology*, VII., 1902, p. 56; XI., 1903, p. 52.

<sup>3</sup> Spaulding, *BIOLOGICAL BULLETIN*, VI., 1904, p. 224.

The sections, the living material and the observations on *Asterias* indicate that the period of great susceptibility is *immediately before and during segmentation* and that just after segmentation great resistance prevails.

In the following table I have placed in parallel columns one of Lyon's results, and the phenomena as shown in sections of the eggs. There is one objection to comparing different series of eggs in that the temperature may not have been the same in the different experiments, and the periods will not exactly coincide. However the eggs of *Arbacia* develop very uniformly and nearly always the first cleavage comes in between fifty and sixty-five minutes after fertilization.

TABLE I.

Minutes After Fertilization.	Susceptibility to Cyanide	Phenomena as Shown in Sections and Living Material
0	Slight.	Egg at rest.
0-10	Slight increase in susceptibility.	Sperm penetrating egg. Aster very small. Meets egg nucleus in 8-12 minutes.
10-25	First period of susceptibility.	Fusion of nuclei. Great growth of aster. Caterpillar stage. Rays throughout cell.
30-48	Progressive increase in immunity. At end most immune.	Pause stage and retrogression of aster. Large rays fade out. Enormous growth of nucleus.
48-60	Second period of susceptibility.	Nuclear wall fades about 45-50 minutes after fertilization. Tremendous growth of asters follows. At 60 minutes chromosomes beginning to separate.
60-65	Susceptible.	Segmentation completed.
65-70	Immune.	Retrogression of asters.

It will be seen from Lyon's experiments that as a rule his period of susceptibility came about fifty-five minutes after fertilization or even five minutes earlier. This period is certainly just before segmentation.

It is clear from this table that the period of great susceptibility coincides with the development of the asters; and the period of greatest immunity coincides with the retrogression of the asters and the development of the nucleus. Thus the first decrease in immunity coincides with the time when the sperm aster is developing. This reaches its maximum about 20-25 minutes after

fertilization. There then ensues a period of retrogression of the aster, the astral radiations fade out, and the nucleus grows enormously. This is the immune period. At the end of that time the nuclear membrane disappears very rapidly and a great growth of the aster takes place, leading up to the first division. This is the second susceptible period. Following segmentation, the nucleus reforms. The asters again decrease in size, and a second period of resistance occurs. This is, however, short, as the second division comes very quickly.

I have attempted to confirm these observations by studying the living *Asterias* egg. I got a very fine lot of *Asterias* eggs, nearly the whole starting maturing within five minutes after shedding. They were fertilized very soon after the germinal vesicle had begun to disappear and transferred at ten-minute intervals (at five-minute intervals during the extrusion of the polar globules and segmentation) to  $m/100$ ,  $m/50$  and  $m/25$  sodium cyanide solutions. After remaining in the cyanide solution for periods of one, two and three hours they were transferred to fresh seawater, which was repeatedly changed, and were then left to develop.

The results were very unsatisfactory. No sharp periods of susceptibility could be discovered in which the majority of the eggs were killed in the two-cell stage as in *Arbacia*. This failure could not be attributed, I think, to lack of uniformity in the development of the egg, since they segmented with marked uniformity. In practically all cases development proceeded after removal of the cyanide, although there were in some cases marked differences in the appearance of the embryos in lots introduced into the cyanide at different times. Those eggs put in the cyanide immediately after segmentation formed swimming gastrulæ which were fairly normal. Eggs introduced into the cyanide immediately before segmentation had the larger number of dwarf and irregular blastulæ and disintegrating eggs. Frequently the eggs after removal from the cyanide did not segment for several hours and then broke at once into a mass of spheres, some of which died, while others formed the embryo.

The resistance of the eggs was remarkable. One series was left for three hours in  $m/25$  cyanide, and the great majority of the

eggs in every lot formed swimming embryos when restored to sea-water.

While the results were thus not sharp and decisive, as in *Arbacia*, and the resistance of the egg to the cyanide very much greater, the experiments were generally harmonious in showing more abnormal embryos in the period just preceding segmentation. In the one exception there were no clear differences between the eggs introduced into the cyanide at different periods.

Why the *Arbacia* eggs are more susceptible when the asters are developing is of course entirely obscure. At this period it is probable, from unpublished observations, that an oxidase escapes from the nucleus and exerts its action upon the cytoplasm. It is possible, also, that at this period the reducing substance in the centriole becomes more active. By an increase in activity is meant an increase in combining power. It may be, therefore, either that the cyanide prevents the oxidase from exerting a necessary action, or it combines with the active centriole and destroys its action, whereas it does not combine with the inactive centriole substance. Further investigation is necessary to decide whether either of these suggestions is the correct explanation. The great resistance of the eggs of *Asterias Forbesii* to the cyanide cannot be explained without further experiment.